TAB 10 Background Information on Biocide Resistance Mechanisms

Sheldon, AT, Jr. Antiseptic resistance: what do we know and what does it mean? ***Clin Laboratory Sci.*** **2005; 18(3):181-187.**

Russell, AD. Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. ***Lancet Infect Dis.*** **2003; 3:794-803.**

Note: Material is provided for background information only; it is not required reading.

Antiseptic Resistance: What Do We Know and What Does It Mean?

ALBERT T SHELDON JR

Biocides (antiseptics, disinfectants, preservatives, sterilants) are used in clinical medicine as intervention strategies that prevent the dissemination of nosocomial pathogens. Biocides are also used for personal hygiene and to prevent cross-contamination of food-borne pathogens in homes, restaurants, day care centers, and nursing homes. However, laboratory evidence has emerged suggesting that the mechanism of nonsusceptibility to biocides may counter-select for resistance to antibiotics. Nature conserves successful survival strategies. Using existing mechanisms of resistance to antibiotics and their means of dissemination, microorganisms have adopted this same survival strategy for biocide nonsusceptibility. These mechanisms are intrinsic in nature or are acquired. The consequences to biocide efficacy in the clinical setting are probably not significant from the biocide perspective. But, the selective pressure biocides exert on bacterial populations that have mechanisms of resistance similar to those to antibiotics or that are also substrates for antibiotic resistance is of concern.

ABBREVIATIONS: CM = cytoplasmic membrane; LPS = lipopolysaccharides; MRSA = methicillin-resistant *Staphylococcus aureus;* OM = outer membrane; PG = peptidoglycan; PMF = proton motive force; RND = resistance-nodulation-division.

INDEX TERMS: biocide, biofilm, efflux, mechanism of action.

Clin Lab Sci 2005;18(3):181

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LEARNING OBJECTIVES

1. Identify the mechanism of action and targets of antiseptics.
2. Discuss the mechanisms of resistance to antiseptics.
3. Describe the intrinsic and acquired mechanisms of antiseptic nonsusceptibility.
4. Discuss the mechanisms used to disseminate resistant determinants of antiseptics.

Semmelweis's mandate that physicians wash examining fingers with chlorine to prevent puerperal (childbed) fever provided the scientific evidence to justify the use of biocides in the practice of medicine.[1] Biocides (antiseptics, disinfectants, preservatives, and sterilants) are now an integral component in the practice of clinical medicine and serve primarily to prevent the dissemination of nosocomial pathogens in the hospital environment.[2] Antiseptics are used as surgical hand scrubs, healthcare personnel hand washes, preoperative skin preparations, and total body washes. Biocides are also used in vascular catheter-care site preparation and are impregnated into catheters to prevent catheter related blood stream infections.[3-5] Disinfectants are used to decontaminate or sterilize medical instruments and patient care items, while preservatives are used to prevent the growth of organisms in multi-use medical products, although not always successfully.[5,7] Biocides are also used in homes, restaurants, day care centers, and nursing homes for personal hygiene and to prevent cross-contamination of food-borne pathogens.[8,9] Intended use of biocides in these settings are not unlike those in the clinical setting: to prevent the dissemination of potential pathogens. However, as with antibiotics, increased use of biocides may contribute to the emergence and/or selection of pathogens less susceptible to biocides and resistant

to antibiotics.[10,11] These observations suggest that antiseptics and antibiotics have common mechanisms of action and possible resistance.

The present article discusses the mechanisms by which biocides exert their biological effect, mechanisms that influence their biological activity, and the possible consequences of these mechanisms in the clinical setting. Although the use of biocides in homes, restaurants, day care centers, and nursing homes is not discussed, the principles discussed regarding the use of biocides in clinical environments are generally applicable to the other environments since their strategic use is the same: the prevention of the dissemination of pathogens.[12-14]

BIOCIDE MECHANISMS OF ACTION

Biocide mechanisms of action are determined using the same methods used in the evaluation of the action of antibiotics. These methods include evaluation of the effects on intracellular components such as interactions with macromolecules and their biosynthetic processes, inhibition of oxidative phosphorylation, and interference with enzymes and electron transport. They also include effects upon membranes such as microscopic examination of cells exposed to biocides; effects on model membranes; and examination of uptake, lysis, and leakage of intracellular components.[15] Since the methods used to assess the mechanism of action include evaluation of their effects on the membrane and intracellular components, these targets are used in our discussion. Although the antimicrobial spectrum of activity and efficacy of biocides is well documented, complete characterization of their mechanisms of action, especially at low concentrations, is lacking. Detailed discussions of the mechanisms of action of biocides are presented elsewhere.[15,16]

The cell wall of gram-positive bacteria is composed of a cytoplasmic membrane (CM), which overlies the cytoplasm and a thick peptidoglycan (PG) outer layer. Gram-negative bacteria add an outer membrane (OM), composed of lipopolysaccharides (LPS), lipoproteins, and proteins, separated from the CM by a periplasmic space.[17]

In gram-negative bacteria, the OM is critical in maintaining the cell wall's integrity as a permeability barrier. The core region of the LPS is negatively charged, impeding permeability and reducing susceptibility to negatively charged antiseptics. Gram-negative bacteria are less sensitive to biocides than gram-positive bacteria because of the LPS layer. Anionic biocides, such as chlorhexidine, neutralize the negative charge and mediate changes in hydrophobicity of the OM thereby promoting uptake. Aldehydes such as glutaraldehyde, interact principally with OM lipoproteins by cross-linking with unprotonated amines resulting in loss of cell wall function. Cross-linking with thiol, sulphydryl, and amino groups also results in inhibition of protein, DNA, and RNA synthesis.[16,18,19]

Biocides also disrupt the CM by dissipating the proton motive force (PMF) of efflux pumps, and interacting with CM enzymes.[16] The PMF is a proton gradient across the CM that develops when the extracellular concentration of protons (H^+) is greater than the intracellular concentration. Efflux pumps use the PMF by coupling biocide efflux to the counterflow of protons.[16] Quaternary ammonium compounds and biguanides are thought to combine with CM phospholipids causing disruption and leakage of intracellular components.[20,21] Biocide mediated inactivation of CM proteins also occurs by inhibition of the electron transport chain and rapid denaturing of proteins.[22,23] Once biocides penetrate the CM, they reach and inhibit the cellular anabolic functions by interacting with DNA, RNA, and proteins.[24,25] The interaction includes cross-linking of thiol, sulphydryl, and amino groups by aldehydes; reactions with cysteine and methionine thiol groups of proteins and nucleotides by iodine; and sulfhydryl groups and double bonds by hydrogen peroxide .[15,16]

Thus biocides, unlike antibiotics, have multiple targets within the microbial cell. This multiple target effect is thought to contribute to their bactericidal activity and dictates against the emergence of resistance. However, recent studies suggest that mutation or overexpression of triclosan and chlorhexidine target sites produces nonsusceptible microorganisms.[14,26-29] These studies suggest that if an antibiotic and antiseptic have a similar mode of action, an organism with reduced susceptibility to the antiseptic may also exhibit resistance to the antibiotic. For instance, in *Escherichia coli* and *Mycobacterium smegmatis*, triclosan binds enoyl-acyl protein reductase, an enzyme involved in fatty acid synthesis.[26] Certain strains of *M. smegmatis* have missense mutations in enoyl reductase genes; they demonstrate decreased susceptibility to triclosan as determined by minimum inhibitory concentration studies and also exhibit resistance to the antituberculosis drug isoniazid.[30] Conversely, a resistant strain originally selected on isoniazid is found to be triclosan non-susceptible. These studies point out a potentially disturbing clinical issue; if both the antiseptic and the antibiotic act on the same target site, then use of either compound may select for and confer resistance to the other.

In prior sections and the remainder of this article, the term nonsusceptible is used instead of resistance to describe the

action of biocides. In the clinical setting, the term resistant is frequently used with antibiotics and suggests that an organism exhibiting this phenotype is likely to result in clinical failure when the antibiotic is used. Currently, biocide susceptibility testing is performed with the methods developed for susceptibility testing of a systemic antibiotic. The interpretation of results may not correlate with the clinical efficacy of the biocide. Thus, to describe a microorganism as resistant to a biocide from susceptibility data derived in this manner does not parallel resistance to a systemic antibiotic. At present, interpretative criteria are not necessary for biocides and topical antimicrobial therapies because the concentrations used in clinical practice are substantially greater than the susceptibilty of pathogens to the biocide or antimicrobial.

In this review and for reasons previously discussed, non-susceptibility to biocides, instead of resistance, is used to describe microorganisms not conforming to the susceptibility patterns of wild-type populations. Although the microorganisms are characterized as nonsusceptible by in vitro testing and molecular methods, microorganisms appear to remain susceptible to approved concentrations of biocides when used as directed in the product label. Regulatory agencies use in vitro and surrogate clinical simulation studies that mimic use conditions to assess the efficacy of topical antiseptics. However, the ability of these surrogate tests to predict efficacy in clinical settings requires validation with clinical trials.[31]

MECHANISMS OF NONSUSCEPTIBILITY TO BIOCIDES

Antiseptic nonsusceptibility mechanisms may be conveniently divided into intrinsic and acquired.[8,15,32]

Intrinsic nonsusceptibility to biocides

Intrinsic nonsusceptibility is mediated by impermeability; efflux, particularly in gram-negative bacteria; biofilms ; and enzyme inactivation. Impermeability is influenced by the composition of the cell wall and physiologic adaptation of the microorganism to its environmant.[8] Among bacteria, biocide sensitivity is based on the permeability of the biocide through the cell wall, gram-positive bacteria being more sensitive to biocides, followed by mycobacteria and gram-negative bacteria, the least sensitive.[33]

Gram-negative bacteria are generally less susceptible to biocides because of their complex cell wall, which is composed of the inner CM and associated efflux pumps, peptidoglycan, and an OM with associated LPS components. The OM also contains hydrophilic channels, porins that regulate the passage of solutes.[34] The main component responsible for the impermeability of the OM is the LPS. Change in cell wall expression or structure leads to increased nonsusceptibility of gram-negative bacteria to biocides.[33] LPS is the primary barrier to the penetration by hydrophobic molecules to the phospholipids and to the cell interior. *Pseudomonas aeruginosa* and *Providencia stuartii* show high-level nonsusceptibility to biocides. This capability may be associated with differences in LPS composition and cation content in the OM, and subtle changes within the structural envelope, respectively.[35,36] In addition, hydrophilic molecules pass readily into gram-negative bacteria but exposure of *P. aeruginosa* and *E. coli* to biocides results in porin loss and subsequent decreased susceptibility to biocides.[34]

Efflux pumps are transporter proteins involved in the removal of toxic substances from the interior as discussed in the companion article on antibiotic resistance. Efflux pumps are found in gram-positive and gram-negative bacteria and are specific for a single drug or substrate while others are capable of transporting multiple substrates. Multidrug efflux pumps showing wide specificity to biocides, dyes, detergents, and antibiotics are found in gram-negative bacteria .[37] In *E. coli*, the Acr AB efflux system belongs to the multidrug efflux system family, resistance-nodulation-division (RND), and acts as a transporter of a range of biocides and antibiotic substrates. Upregulation of *acrAB* is mostly a property of the multiple antibiotic resistance activator (MarA). Environmental stimuli can increase expression of MarA resulting in elevated levels resulting in nonsusceptibility.[37] Biocides such as pine oil stimulate reduced susceptibility not only to pine oil but also to clinically useful antibiotics. Mutations found in the multiple antibiotic resistance repressor (MarR), allow expression of MarA and activation of the efflux pump acrAB resulting in reduced susceptibility not only to pine oil but also to triclosan.[37,38]

Physiologic adaptation resulting in nonsusceptiblity to biocides is usually encountered as a biofilm in the clinical setting especially with indwelling medical devices or contaminated products.[39] A biofilm is a microbiological community of sessile organisms irreversibly attached to a surface and embedded in a self-produced polymeric extracellular matrix. The organisms of a biofilm exhibit an altered growth rate.[39] The nonsusceptibility of bacteria in biofilms to biocides is caused by numerous factors including nutrient depletion within the biofilm resulting in altered

growth rates, binding of the biocide to the biofilm, and neutralization or degradation of the biocide.[39]

Degradation or inactivation, via enzymatic mechanisms, has been reported for formaldehyde, chlorhexidine, and quaternary ammonium compounds but at concentrations below those used in clinical practice.[8,12] Thus the clinical significance of this mechanism may be its importance in selecting bacterial species capable of hyperexpressing these enzymes and serving as reservoirs for their dissemination if plasmid mediated.

Acquired nonsusceptibility to biocides

Acquired nonsusceptibility to biocides can occur by mutation of target site, overexpression of the target site, and plasmid mediated efflux.[8,9,15]

In gram-negative bacteria, studies that describe changes in permeability leading to acquired biocide nonsusceptibility suggest target site mutation.[38] Although the changes leading to biocide nonsusceptibility have not been fully characterized at the genetic or molecular level, the phenotypic observations described suggest changes in the outer membrane fatty acid and protein composition, ultrastructure, and surface hydrophobicity.[19,39]

Studies with triclosan, a bis-phenol found in many products, describe a defined target site, and by mutation or hyperproduction of this site, non-susceptible microorganisms are isolated.[14,40-42] In *E. coli,* triclosan binds enoyl-acyl protein reductase (Fab1), an enzyme involved in fatty acid synthesis.[43] A similar mechanism of action is described for *M. smegmatis*, where strains with enoyl reductase missense mutations have decreased susceptibility to triclosan and resistance to the antituberculosis drug isoniazid.[30] Conversely, the same study found that a resistant strain originally selected on isoniazid was also triclosan non-susceptible. These studies provide evidence that the antiseptic and the antibiotic act on the same target site and the emergence of resistance to one compound counter-selects for resistance to the other compound.

As previously discussed, efflux pumps can mediate intrinsic nonsusceptiblity to biocides. In addition, studies reveal a mechanism of enhanced nonsusceptibility to antiseptics mediated by overexpression or mutation of regulatory regions of genes of multidrug efflux pumps.[44-46] Mutation of the repressor/operator region controlling efflux pump gene expression (*MarA*), or mutation of the efflux pump structural gene, results in either enhanced efflux or reduced affinity to the antiseptic for the efflux pump.[44,45] Efflux is responsible for low-level nonsusceptibility to cationic biocides in antibiotic resistant cocci and in gram-negative bacteria.

Plasmid-associated nonsusceptibility in staphylococci has been demonstrated for cationic biocides such as chlorhexidine gluconate and quaternary ammonium compounds.[11,15] *S. aureus* and coagulase-negative staphylococci isolated from human and veterinary sources were evaluated and shown to carry multidrug resistant plasmids conferring nonsusceptibility to biocides and antibiotics.[47-49] The *qacA, B, C,* and *D* genes encoding multidrug efflux pumps mediated the nonsusceptibility. The multidrug resistant determinants *qacA-G* encode proton-dependent export proteins and have significant homology to other energy dependent transporters such as those found in tetracycline exporter mediated resistance.[12] Although evidence of plasmid-borne biocide resistance in gram-negative bacteria is limited, plasmid-encoded changes suggest alterations of the OM proteins, and composition of the OM LPS and reduced expression of porins.[12]

Consequences of reduced susceptibility to biocides

The nonsusceptibility of microorganisms to biocides and the targets some biocides share with antibiotics is of clinical concern because antibiotics are important armaments in the treatment of disease. The concern is primarily the use of biocides in non-clinical environments and the impact such use has on the selection of pathogens cross-resistant to therapeutically useful antibiotics.[10,14,50] There are two distinct issues that arise from these observations. The first is whether the development of nonsusceptibility to biocides by nosocomial pathogens, skin flora, and other microorganisms results in decreased efficacy of the topical biocides used in homes, restaurants, day care centers, nursing homes, and healthcare settings. Biocides, when used as disinfectants and sterilants, are used at concentrations substantially higher than levels required to show bactericidal effects. Concentrations of skin antiseptics and preservatives, although lower than disinfectants and sterilants, also demonstrate bacteriostatic and bactericidal effects versus nonsusceptible vegetative pathogens. Although mechanisms resulting in nonsusceptibility to biocides are observed in laboratory studies, clinical evidence has not emerged that combinations of intrinsic and acquired mechanisms of nonsusceptiblity result in clinical failure of biocides when used at recommended concentrations.[51] However, we must consider that biocide concentration decreases to sub-therapeutic concentration as we progress away from their point of use and this may provide the environment and selective pressure for nonsusceptible microorganisms.

The second issue is the consequence to the medical community of biocides that select for nonsusceptible microorganisms that are cross-resistant to antibiotics.[10,11,14,51] If biocide nonsusceptible organisms that are cross resistant to important antibiotics emerge in clinical and domicile environments, we create an undesirable outcome: a microorganism that may not be treatable in the clinical setting. The use of the biocide triclosan in the domicile environment may explain the emergence of community acquired methicillin-resistant *S. aureus* (caMRSA).[10,14] The emergence of caMRSA is not associated with the risk factors normally seen in the emergence of antibiotic resistance and suggests that other, previously unidentified risk factors, such as use of triclosan, may be responsible.[51] Since triclosan is also used in the clinical environment, the in vitro susceptibility of methicillin-resistant *S. aureus* and *S. epidermidis* to triclosan suggests that MRSA isolates do not have higher minimum inhibitory concentrations (MICs) to triclosan when compared to wild-type populations. However, *S. epidermidis* does, suggesting a possible association between the use of triclosan and selection for a nonsusceptible subpopulation.[52,53] Thus, the in vitro observations do not support the proposed hypothesis.

Clearly, nature is conservative in the application of strategies that enhance survival of living organisms. Therefore, it is reasonable to expect that existing survival strategies, e.g., antibiotic resistance, may be applicable to other toxic molecules, e.g., biocides encountered by microorganisms. Mutants of *M. smegmatis*, whether selected on triclosan or isoniazid, showed cross-resistance to both drugs via mutation of the *inh*A gene.[30] The published literature suggests that microorganisms adapt the same strategies in dealing with the toxic effects of antibiotics and biocides. For example, the same mechanisms that mediate resistance to antibiotics, i.e., efflux, changes in target site, and impermeability are the mechanisms used to produce nonsusceptibility to biocides.[8,12,15,30,40]

In addition, mechanisms mediating nonsusceptibility by efflux i.e., *qacA-G,* are found on plasmids; the same evolutionary strategy used by bacteria to disseminate antibiotic resistant determinants.[48,49] From the pathogens' perspective, the acquisition of plasmids mediating biocide nonsusceptibility and antibiotic resistance is a desirable survival strategy. These parallels between nonsusceptibility to biocides and antibiotic resistance demonstrate that evolution is a conservative yet dynamic process and when successful strategies evolve, microorganisms adapt these strategies to counter toxic environments. Thus, it is logical that if the mechanism of action of the antibiotic and the antiseptic are the same, cross-resistance is likely to occur. In addition, if the biocide and antibiotic resistant determinants are resident in the same host, then exposure of the host to either the biocide or the antibiotic counter-select for the other mechanism.

There is concern that inappropriate use of biocides may result in the selection of antibiotic resistant pathogens. Increased selection pressure by antibiotics and biocides will result in population shifts to less susceptible organisms. But, we must also realize the importance of biocide use in the clinical and domicile environments. Semmelweis documented the importance of antiseptics in clinical medicine; the importance of biocides in the domicile environment remains to be determined. The current debate appears to focus on the justification for the prevalence of biocide containing products in domicile environments and the consequences such uses may have on selection of antibiotic resistant resident and transient microorganisms. Laboratory studies have shown the potential for cross-resistance between antiseptics and some antibiotics, prompting professional organizations to question the benefit of antimicrobial impregnated household products, and to warn of potential for the emergence of antiseptic mediated resistance to useful antibiotics.[54,55] Implied in this concern is acknowledgement that biocides are an important and critical component of the practice of medicine and the healthcare community. As with antibiotics, we must use biocides in a conservative and beneficial manner to assure their continued usefulness.

CONCLUSION

Biocides are an integral and necessary component of the clinical strategy used to prevent the dissemination of nosocomial infections in the clinical community. Their efficacy is well documented. Unlike antibiotics, the mechanism of action of biocides remains poorly characterized. The published literature accepts that biocides have multiple target sites with use concentrations resulting in bactericidal effects. However, the use of subtherapeutic concentrations may allow the identification of specific targets. Characterization of the target sites is necessary to understand whether single target sites exist and the relationship of these targets in the selection of resistance to important antibiotics. In addition, surveillance studies are needed to understand the prevalence of mechanisms of nonsusceptibility to biocides in the microbial community. By applying the same epidemiological tools used to monitor antibiotic resistance to monitor changing susceptibility patterns to biocides, we can then make reasonable risk/benefit decisions regarding the potential implications of biocide use and the emergence of antibiotic resistance.

Albert T Sheldon Jr is an employee of Antibiotic and Antiseptic Consultants Inc and as such has no financial holdings and receives no financial support from any manufacturers. The views and opinions expressed in this publication are the author's.

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Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations

A D Russell

Antibiotics are used as chemotherapeutic drugs, and biocides are used as antiseptics, disinfectants, and preservatives. Several factors affect biocidal activity, notably concentration, period of contact, pH, temperature, the presence of interfering material, and the types, numbers, location, and condition of microorganisms. Bacterial cells as part of natural or artificial (laboratory) biofilm communities are much less susceptible than planktonic cells to antibiotics and biocides. Assessment of biocidal activity by bactericidal testing is more relevant than by determination of minimum inhibitory concentrations. Biocides and antibiotics may show some similarities in their mechanisms of action and common mechanisms of bacterial insusceptibility may apply, but there are also major differences. In the laboratory, bacteria can become less susceptible to some biocides. Decreased resistance may be stable or unstable and may be accompanied by a low-level increase in antibiotic resistance. Laboratory studies are useful for examining stress responses and basic mechanisms of action and of bacterial insusceptibility to antibacterial agents. Translation of such findings to the clinical and environmental situations to provide evidence of a possible relation between biocide use and clinical antibiotic resistance is difficult and should be viewed with caution.

Lancet Infect Dis 2003; **3:** 794–803

Antibiotics are used to treat bacterial or fungal infections in human beings and animals. Biocides encompass chemicals with antiseptic, disinfectant, and/or preservative activity.[1,2] They are used for a range of purposes, usually with inanimate objects (hard surface disinfectants), externally on the skin (antiseptics and topical antimicrobials) to prevent or limit microbial infection, for preoperative skin disinfection, or incorporated (preservatives) into pharmaceutical, cosmetic, or other types of products to prevent microbial contamination (table 1). Some agents, for example chlorhexidine salts and quaternary ammonium compounds (QACs), are used for all three purposes (antisepsis, disinfection, and preservation) whereas others (glutaraldehyde, orthophthalaldehyde) are used predominantly for the disinfection of endoscopes.[1]

To survive in the environment, bacteria and other microorganisms must respond to several stresses such as low nutrient concentrations and non-ideal growth conditions. As an additional stress, they may be exposed to a wide range of antibiotics and biocides that could act as a selective pressure for the development and isolation of resistant cultures by several mechanisms.[4] This article will explore whether (1) biocide use could lead to the development or induction of a coping mechanism that results in new or increased antibiotic resistance,[5] and (2) the findings from laboratory tests, in which many stresses are controlled, are relevant to the clinical and environmental situations where those same stresses are uncontrolled. The last aspect has not received the attention that it merits.

Table 1. Some types of clinical and other uses of biocidal agents

Biocide type	Example(s)	General examples of use(s)
Alcohols	Ethanol	Hand sanitising
Aldehydes	Formaldehyde	Virucidal agent
	Formaldehyde-releasing agents	Topically; irrigation solutions
	Glutaraldehyde	Endoscope disinfection
	Orthophthalaldehyde	Endoscope disinfection
Biguanides	Chlorhexidine	Antiseptic, disinfectant, pharmaceutical preservative
	PHMB (polymeric)	Swimming pool disinfection, contact lens solutions
CRAs	NaClO, NaDCC industria	Disinfection of blood spillages; sanitisation compounds
Isothiazolones	Chloromethyl and methyl derivatives	Preservatives (cosmetics, pharmaceuticals)
Peroxygens	Hydrogen peroxide	Antiseptic, disinfectant, deodorant
	Peracetic acid	Endoscope disinfectant
Phenylethers	Triclosan	Body washes, dental hygiene
Phenols and cresols		Preservatives, disinfectants
QACs	BZK, CPC, Cetrimide/cetylpyridinium	Skin disinfection; peoperative disinfection; antisepticis; pharmaceutical preservatives
Vapour phase	Ethylene oxide	Low temperature sterilisation of thermostable materials

PHMB=polyhexamethylenebiguanide; NaClO=sodium hypochlorite; NaDCC=sodium dichlorocyanurate; BZK=benzalkonium chloride; CTAB=cetyltrimethylammonium bromide.

Terminology

Whereas the terminology pertaining to antibiotic action and resistance is well understood, that relating to biocide activity, and especially to biocide resistance, is still the subject of debate.[6] By analogy with antibiotic resistance, a

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culture is considered to be resistant to a biocide when it is not inactivated by an in-use concentration of a biocide, or a biocide concentration that inactivates other strains of that organism. Other terms that have been suggested to describe the decrease of biocide susceptibility in laboratory culture include the two terms used here ("insusceptibility", "reduced susceptibility"), "tolerance", and "tolerant".

Factors affecting biocide activity

Biocide activity is affected by several factors—notably concentration, period of contact, pH, temperature, the presence of organic matter or other interfering or enhancing materials or compounds, and the nature, numbers, location, and condition of the microorganism (bacteria, spores, yeasts and moulds, protozoa) or entities (prions, viruses). Concentration is a factor of prime importance.[1,7] The concentration exponent (n or η) measures the effect of concentration, or dilution, on the activity of a biocide. Biocides with high η-values—eg, phenolics, alcohols—rapidly lose activity on dilution, whereas those with low η-values (QACs, chlorhexidine, glutaraldehyde, orthopthalaldehyde) retain much of their activity on dilution.[7] This difference is important when assessing lethal activity but is also of significance in clinical and other environments where biocide residues must be considered.[8]

Many biocides have an optimum pH range of activity.[1] Glutaraldehyde and cationic biocides (chlorhexidine, QACs) are most active at alkaline pH, whereas hypochlorites and phenolics are most potent at acid pH. The activity of biocides increases at raised temperatures, but this activity now finds little practical use. Interaction with organic matter (as blood, serum, pus, dirt) and non-ionic surfactants, and adsorption to containers and closures can adversely affect the efficacy of many biocides. Microorganisms and entities (prions, viruses) show considerable differences in their response to biocides,[6] and their condition—eg, as biofilm cells—has a marked outcome on biocide susceptibility.

The activity of biocides and antibiotics against Gram-negative organisms may be enhanced by permeabilisers, chemical agents that increase the permeability of a bacterial cell.[9] The best-known example is ethylenediamine tetra-acetic acid (EDTA), which chelates divalent cations from the outer membrane, especially *Pseudomonas aeruginosa*. Other examples include polylysine and polyethyleneimine which act by displacing cations.[9] Activity can also be enhanced by (1) using a combination of biocides (or of antibiotics), or (2) combining an efflux inhibitor with an antibacterial compound. While the last approach might operate in vitro, caution is needed in the clinical situation because of possible toxicity of the efflux inhibitor to human or animal cells. The composition of biocide formulations must be considered[10] because other constituents might themselves possess antimicrobial activity or potentiate (or sometimes modify) biocide activity.[1] Thus, formulations at recommended in-use dilutions should be tested as well as "pure" compounds, since the activity of the former should never be based solely on studies with the latter.

Assessment of antibacterial activity of biocides and antibiotics

Minimum inhibitory concentrations (MICs) have typically been used to examine bacterial sensitivity to antibiotics. Standard methods relate disc sensitivity to MICs.[11] Although many antibiotics (β-lactams, aminoglycosides-aminocyclitols [AGACs]) are bactericidal rather than bacteriostatic (tetracyclines, chloramphenicol), MICs have provided a convenient way of relating sensitivity with (usually) blood serum or tissue concentrations of an antibiotic after oral or parenteral administration. Serum binding should also be assessed. Minimum bactericidal concentrations (MBCs) can be established from MIC experiments by subculturing from growth-negative media into fresh drug-free media. This process does not provide a true quantitative picture, and is certainly not satisfactory with biocides.

Methods used to assess antibiotic resistance can lead to inappropriate conclusions if applied to biocides.[12,13] With biocides, MICs provide a useful starting point only but can be related to preservative use in which prevention of bacterial/microbial multiplication and reduction of viability to official levels are more appropriate than inactivation. However, biocides used as antiseptics or topical antimicrobials, and especially as disinfectants, are usually used at concentrations well in excess of MIC values, although MICs of triclosan against *P aeruginosa* approach the levels of the phenylether used in practice.[14,15] Thus, tests of their lethal effects, and of the factors affecting efficacy, must be undertaken in the laboratory and under simulated and actual conditions of use (figure 1).[16] At present, there are few publications that have examined the association between triclosan use and antibiotic resistance in the clinical setting. Some of these publications are based on

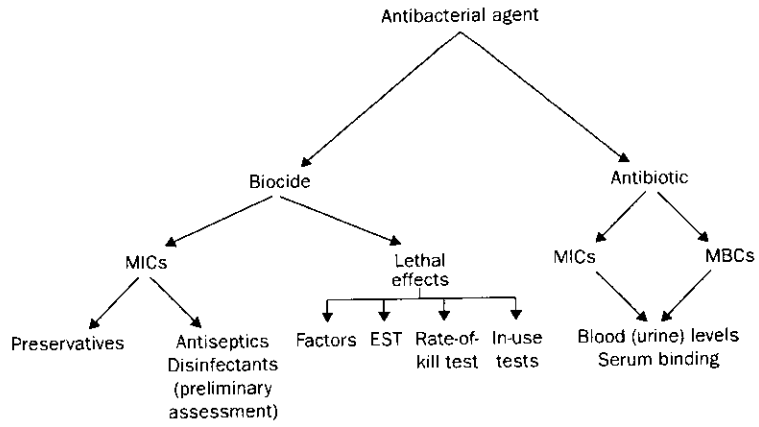

Figure 1. Determination of antibacterial activity of biocides and antibiotics. MIC=minimum inhibitory concentrations. MBCs=minimum bactericidal concentrations. EST=European suspension test.

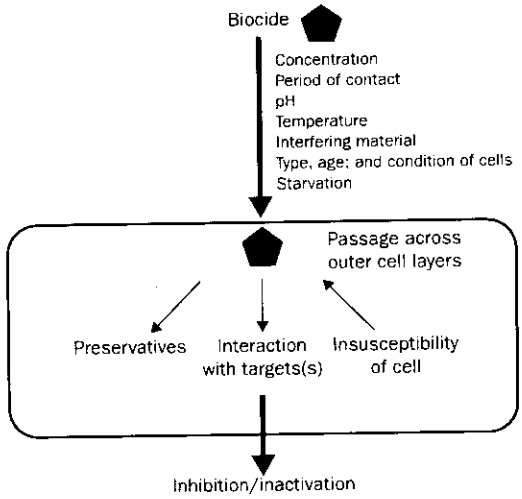

Figure 2. Bacterial responses to a biocide, showing the possible outcomes.

small numbers of isolates with limited data on antibiotic or triclosan exposure rates in the patients from which the isolates had been obtained,[17-20] altough more comprehensive data, to be considered later, were provided by Al-Doori et al.[21]

Response of bacteria to inimical (hostile) agents

Stress can be defined in different ways, each of which has certain limitations or disadvantages.[22] Thus, stress can be (1) any deviation from optimum growth conditions that results in a reduced growth state, although some adaptive or stress responses function so well that growth is not impaired; (2) exposure to any environmental situation that results in damage to cellular components in the absence of a cellular response; or (3) a situation that stimulates the expression of genes known to respond to a specific environmental condition. The so-called heat-shock proteins that are produced when bacteria are subjected to high temperatures and to at least some chemical agents provide an example of the synthesis of new gene products. However, there may be novel stress conditions under which previously unidentified sets of genes are induced.[23] Oxidative stress is a disturbance in the pro-oxidant-antioxidant balance in favour of the former. There is evidence of a regulated adapted response in growing *Escherichia coli* cells exposed to hydrogen peroxide, with the cells becoming resistant to normally lethal doses of peroxide and the synthesis of around 40 new proteins.[8]

When exposed to a harmful stress, bacteria will do all in their power to survive. The effects of an inimical agency on bacteria (figure 2) can be seen as producing a stress response, causing inhibition or inactivation of the cell, or resulting in tolerance/resistance of the cell. In the natural environment, microorganisms exist under conditions that might support only slow growth.[17] Rapid environmental changes are also likely to occur so that a normal lifestyle involves exposure to constantly changing stresses.

Biocide and antibiotic action: similarities and differences

Most antibiotics inhibit a specific target in a biosynthetic process. Selective toxicity arises because the process (bacterial peptidoglycan synthesis) is absent, or differs significantly (protein, DNA, RNA syntheses) from a similar process in host cells. By contrast, biocides have multiple, concentration-dependent targets, with subtle effects occurring at low concentrations and more damaging ones at higher concentrations.[7]

Nevertheless, some similarities have been described (table 2). These similarities include the penetration of cationic agents, both biocides and antibiotics, into Gram-negative bacteria;[9] entry by passive diffusion into non-mycobacterial non-sporing Gram-positive bacteria;[9] entry into mycobacterial cells;[7] membrane-damaging effects produced by some biocides and antibiotics;[24,25] similar morphological changes;[25] and a shared target site between one biocide (triclosan) and a chemotherapeutic drug, isoniazid (isonicotinyl acid hydrzine) in some mycobacteria.[26]

Table 2. Similarities between biocide and antibiotic action

Property or effect	Process	Biocides	Antibiotics
Uptake into			
(1) Gram-negative bacteria	Displacement of OM divalent cations	Cationic: CHX, QACs	AGACs, polymyxins
(2) Gram-positive cocci	Passive diffusion	Most?	Most?
Damage to CM	Disruptive effect	CHX, QACs, alcohols, phenolics, triclosan	Polymyxins, streptomycin
Inhibition of synthesis	PTG	?	β-lactams, vancomycin
	Protein	Parabens	Chloramphenicol, Tetracyclines, Fuc
	RNA	Parabens, PEA, POE	Rifampicin
	DNA	Parabens, PEA, POE	Fluoroquinolones
Specific enzyme inhibition	Enoyl reductase		
	E coli	Triclosan	?
	M smegmatis	Triclosan	Isoniazid
	Thiol groups	Heavy metals, isothiazolones	?
Effects on DNA	Interaction/ intercalation	Acridines, CHX, ACs, Ag^+	Mitomycins, actinomycin D
	Enzyme inhibition (gyrase)	?	Fluoroquinolones novobiocin
Cytological effects	Filament formation	Acridines, PEA, POE, chloroacetramide	β-lactams, fluoroquinolones, novobiocin

CM=cytoplasmic membrane; OM=outer membrane; CHX=chlorhexidine salts; QACs=quaternary ammonium compounds; parabens=esters of para (4) -hydroxybenzoic acid; PEA=phenethyl alcohol; POE=phenoxyethanol; AGACs=aminoglycoside-aminocyclitol group; Fuc=sodium fusidate; ?=unproven or not yet found.

Furthermore, at low concentrations, biocides may be much more selective in their action than when used at higher, in-use levels.[9,21,27] This possibility shows the need for studying biocides at the low, residual concentrations that could remain on surfaces or other materials,[27,28] with an experimental approach described previously.[8]

Bacterial resistance and insusceptibility to biocides and antibiotics

Basic mechanisms

Similarities and differences exist in the manner in which bacteria resist the action of biocides and antibiotics (figure 3). Intrinsic resistance (intrinsic insusceptibility) is a natural property of an organism. It is usually shown (table 3) as a reduced uptake of an antibiotic or biocide and occurs as a result of impermeability barriers[5] in bacterial spores, mycobacteria, Gram-negative bacteria, and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains.[28,29] Vancomycin resistance arises by mutation and cell-wall thickening.[29] Enterococci are less susceptible to biocidal action than are staphylococci,[1,30] possibly because of a lower cellular uptake. Resistance can also occur as a result of efflux pumps that effectively remove toxic compounds from cells,[31,34] although their efficacy will depend on antibiotic/biocide concentration. Some biocides can induce efflux even though they are not substrates.[33] Phenotypic adaptation to intrinsic resistance may be shown by biofilm cells, as described later.

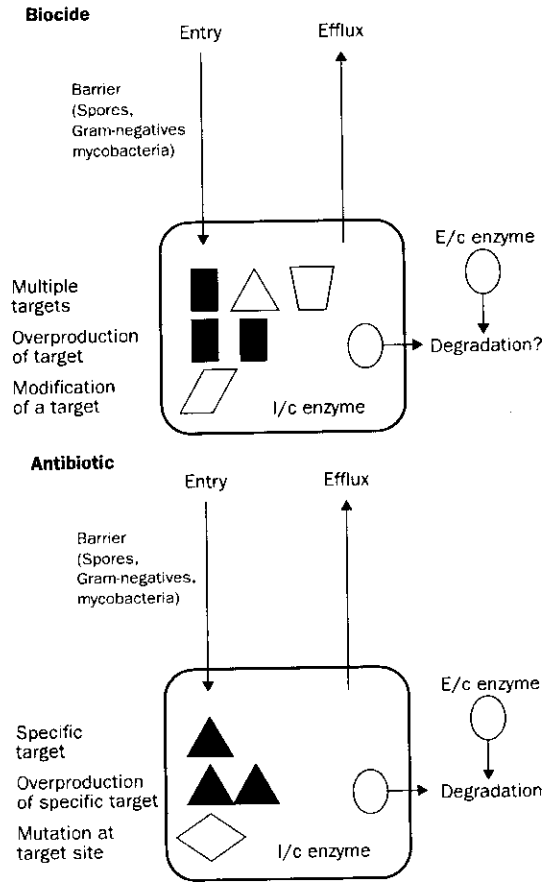

Figure 3. Mechanisms of bacterial resistance or insusceptibility to biocides and antibiotics, showing possible similarities (eg, permeability barrier, efflux) and differences (eg, single or multiple targets). Degradative enzymes—eg, β-lactamases—are often of significance in antibiotic resistance but are unlikely to be so with in-use biocide concentrations.

Acquired insusceptibility (table 3) to biocides and especially to antibiotics may arise by mutation or adaptation or by the acquisition of plasmids, transposons, or other genetic elements.[12] Mutational resistance to antibiotics is a well-known event.[1] Target-site mutations are rare with biocides.[33] It is unlikely that mutation to high-level resistance occurs with biocides, which usually have a multiplicity of actions.[33] However, at low concentrations, triclosan inhibits a specific enzyme, enoyl reductase, in *E coli*[35] and other bacteria;[36] mutation to produce an altered enzyme or overexpression of the gene can produce resistance to this agent. Highly specific mutations confer antibiotic resistance, as exemplified by an altered penicillin-binding protein 2 in meticillin-resistant *S aureus* (MRSA).[37] Other specific mechanisms include enzymatic inactivation (β-lactams, erythromycin, tetracyclines, and chloramphenicol) or modification (AGACs), duplication of the target site with the second version being less susceptible (dihydrofolate reductase and trimethoprim resistance), overproduction of target, and the absence of a specific metabolic pathway.

Stability of resistance or insusceptibility of laboratory cultures

Exposure of pure bacterial cultures under laboratory conditions to a biocide (or antibiotic) may result in a loss of susceptibilty. Typically with antibiotics, "stepwise training" methods have been used in which bacteria are gradually exposed to increasing concentrations of the test drug. Laboratory training may be criticised on the grounds that a similar event would be unlikely to occur in practice. Concentrations in practice may vary considerably and it is not inconceivable that resistance to low concentrations will enable organisms to obtain some degree of resistance that will be enhanced when next they meet that particular drug. In other laboratory procedures (figure 4), single colonies have been picked off from within inhibition zones surrounding antibiotic discs, or isolated colonies have been removed from the surface of plates containing an inhibitory antibiotic concentration onto which a dense inoculum has been evenly spread. Resistance may be lost if the organisms are repeatedly grown in medium lacking the selective drug.

Such methods have met with varying degrees of success with biocides. *Pseudomonas aeruginosa* has been trained to become even less susceptible to cationic biocides by being exposed to gradually increasing concentrations of chlorhexidine or QACs.[8] *Pseudomonas stutzeri*, which is inherently more sensitive to biocides, could be trained to insusceptibility to these agents.[17,40] Strains of *S aureus* with reduced susceptibility to triclosan have been produced by stepwise training and, despite some initial problems,[17] by isolation of colonies from within disc inhibition zones.[18]

Table 3. Mechanisms of bacterial resistance to antibiotics and biocides

Mechanism of resistance	Example(s)
Intrinsic	
Reduced uptake	
1) Impermeability:	
G-ves	*P aeruginosa*: triclosan, chlorhexidine, QACs
Spores	*Bacillus subtilis*: chlorhexidine, QACs
Mycobacteria:	*Mycobacterium chelonae*: chlorhexidine, QACs
Staphylococcus aureus	VRSA, VISA/GISA (thickened cell walls): phenols
(2) Efflux	MDR G-ves: several biocides and antibiotics
Inactivation*	Some β-lactams, triclosan?, chlorhexidine?
Acquired	
Altered target site	Triclosan, β-lactams, tetracyclines, rifampicin, trimethoprim, vancomycin (VRSA, VISA/GISA)
Inactivation	Some β-lactams, chloramphenicol, erythromycin, formaldehyde
Modification	AGACs
Efflux	Several ABs; *qac* genes and cationic biocides
Bypass of sensitive step	Sulphonamides, trimethoprim
Overproduction of target	Trimethoprim, triclosan
Absence of enzyme-metabolic pathway	Isoniazid (in *Mycobacterium tuberculosis*)

*Inactivation of triclosan and chlorhexidine not shown to be a major resistance mechanism. G-ves=Gram negative bacteria; QACs=quaternary ammonium compounds; AGACs=aminoglycosides-aminocyclitols; MDR=multidrug resistance.

The latter method is not effective with biocides such as chlorhexidine and QACs, which diffuse poorly in agar. On removal of biocide, reduced susceptibility may either be retained or lost (figure 4), depending on the nature of the biocide and the type of cells.

Reduced susceptibility and insusceptibility to biocides in practice

Populations of bacteria with reduced susceptibility to biocides sometimes arise in practice, but usually indicate a capacity for phenotypic adaptation and survival in a constantly changing environment and where conditions are stressful and growth-limiting.[11] Susceptibility may be restored when the biocide is withdrawn.

Bacterial isolates from industrial or clinical sources may show decreased susceptibility to biocides compared with "standard" (culture collection) strains, although in some of the earlier studies it was not realised that MIC values alone did not provide a suitable evaluation procedure.[42] There are several possible reasons for this reduced biocide susceptibility (figure 5)—notably (1) the presence in the environment of biocide residues, to which bacteria could develop low-level insusceptibility,[8] (2) the deplored practice of "topping-up" of biocide solutions (which could lead to the employment of inadequate concentrations) in hospitals, (3) the incorrect use of biocides—eg, in "dirty" situations—and (4) the use of a biocide that is ineffective against the likely contaminant for an inadequate period of time.

MICs of triclosan versus *P aeruginosa* fall within the range of in-use concentrations of the phenylether.[14,15,43,44] For many organisms, however, MICs are considerably below in-use concentrations.[7,12,13,17,42,45] Of greater relevance than MICs are lethal concentrations, but these also are generally well below in-use levels.[17,18,45]

Levy[14] is of the opinion that, as with antibiotics, biocide resistance will eventually emerge on a major scale. So far there have been no reports of environmental outbreaks of such resistance, despite the use of many biocides for many years.[13] There are, however, indications of changes in species prevalence in both the clinical[45,47] and in-vitro[48] settings. For example, the use of triclosan to curb an MRSA outbreak resulted in the dominant species becoming *P aeruginosa*.[46]

Clinical antibiotic resistance

There are several reasons for the failure of antibiotic therapy (figure 6). These are overuse of antibiotics, incorrect or inadequate prescribing, non-completion of prescribed courses of therapy (especially in the treatment of tuberculosis where prolonged treatment is necessary), and incorporation into animal feeds of antibiotics identical with or similar to drugs used in human or animal therapy.[49–51]

It has also been claimed that widespread biocide use in hospital, domiciliary, industrial, and other settings contributes to the overall rate of drug resistance.[1] Significantly, however, strains isolated from a hospital pharmacy unit in which biocides are used to some degree differed in type from those in an intensive care unit (ICU)

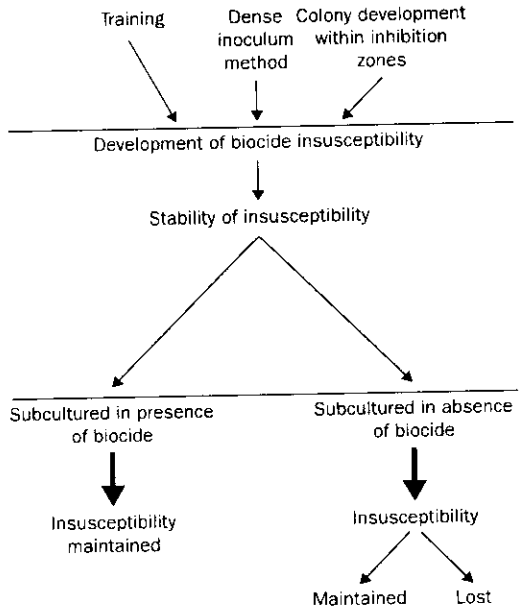

Figure 4. Development of biocide resistance and stability or loss in laboratory cultures.

and were more sensitive to antibiotics and biocides.[45] In ICUs, there is a preponderance of seriously ill patients and antibiotics are widely prescribed, so that there is an intensive selective pressure for antibiotic resistance.

Biofilms—sessile versus planktonic cells

A biofilm is a microbially derived sessile community characterised by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polysaccharide substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.[52] A vital element in bacterial infections, including those related to indwelling medical devices, is attachment of organisms to surfaces.[53] Sessile bacteria contained within biofilms are much less readily inactivated by antibiotics or biocides than are planktonic cells in liquid culture.[54–56] There are several reasons, additional to those basic mechanisms already described, for this increased resistance. Biofilms are highly structured habitats with spatial heterogeneity accompanied by physiological heterogeneity that develops at a phase interface.[57] Genetic exchange can take place and organisms can deposit enzymes such as β-lactamases and proteases that can hydrolyse β-lactams and possibly some biocides within the matrix. Quorum sensing involving cell-to-cell signalling is an important feature of biofilm regulation.[57,58]

Early stress responses (figure 2, planktonic cells) that involve the activation and expression of new groups of genes may be involved in the survival of biofilm cells exposed to biocides.[9,59] Diffusion of antibiotics and biocides and possible interaction with biofilm constituents, which controls penetration, must be considered. A biocide concentration gradient is produced. In a thick biofilm there will be a use-concentration at the surface but a decreased concentration as the antibacterial agent penetrates into the community.[59] Degradative enzymes that might have only a minor role in the insusceptibility of planktonic bacteria to biocides (table 3) would thus be expected to be more effective with sessile cells against these reduced concentrations. Slow-growing deeply recessed bacteria with a less susceptible phenotype are subjected to a lowered biocide concentration.[59] The importance of biocide concentration was emphasised above.

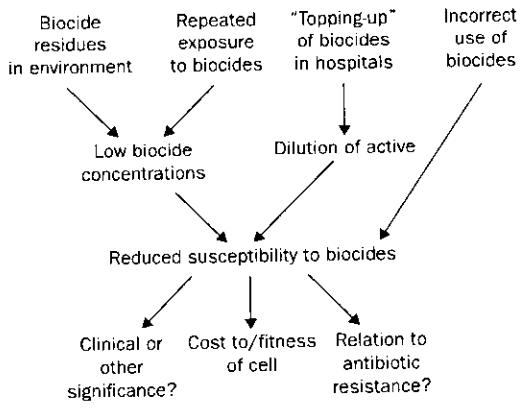

Figure 5. Reduced biocide susceptibility and possible significance to antibiotic resistance clinically or in the environment.

In addition to these chemical gradients, physiological gradients also apply. For example, nutrients and oxygen will be consumed at the periphery of biofilms, whereas cells deeply placed within the community are starved of both. Nutrient-limited cells expressing starvation phenotypes are more resistant to biocides than are "normal" cells.[60] Additionally, pockets of surviving organisms may occur as small clusters, although neighbouring cells have been inactivated.[61] These clusters might include efflux mutants as well as genotypes with modification in single gene products. Clonal expansion after exposure to a sublethal concentration could result in the emergence of a population resistant to antibiotics but less likely to be resistant to biocides with multiple target sites.[9,47]

Sublethal treatment could also induce the expression of multidrug efflux pumps. Thus, *mar* expression is greatest within the depths of a biofilm where growth rates are at a minimum, but neither *mar* nor *acrAB* is specifically induced within biofilms.[62,63] Persisting cells (persisters) have been suggested as forming part of a programmed cell death (PCD) whereby inactivation of biocide-treated cells arises from a programmed suicide mechanism and cell lysis.[64,65] Persisters are cells defective in PCD that will grow rapidly in the presence of exudate released from lysed community cells. In *P aeruginosa* biofilms, only about 1% of the genes showed differential expression in the two growth modes (planktonic, sessile) and about 0·5 % of the genes were activated and about 0·5% repressed.[66] A cycle of resistance development may occur with biocide-treated biofilms. Not all the cells are inactivated so that, after a period of recovery, resistant clones become enriched and less sensitive bacteria are selected when the treatment process is repeated.[59]

Laboratory-generated biofilms have provided useful data as to how biofilms can arise in nature, for example with indwelling medical devices such as central venous and urinary catheters, and of possible ways of preventing biofilm formation and treating biofilm cultures.[24,52,53]

Biocide use and antibiotic resistance

Laboratory studies

Laboratory studies have shown that bacteria can become less susceptible to a biocide, that this may be stable or unstable and that crossresistance may occur to other biocides and to antibiotics.[11,17,18,37,40] Efflux proteins in *P aeruginosa* have been widely studied and shown to be associated with some antibiotics and biocides.[14] A small multidrug resistance family protein (EmrE) encoded by the *emrE*$_{Pae}$ gene in *P aeruginosa* has been described.[67]

Standard strains of *S aureus* and *E coli* are highly susceptible to chlorhexidine and QACs and it is difficult to produce less susceptible subcultures, whereas strains of the normally highly sensitive *P stutzeri* can be produced that are markedly less susceptible to chlorhexidine and to a QAC, cetylpyridinium chloride. These show crossresistance to some other biocides and to some antibiotics,[38,40] possibly as a result of outer membrane changes producing a blanket, non-specific increase in cell impermeability.

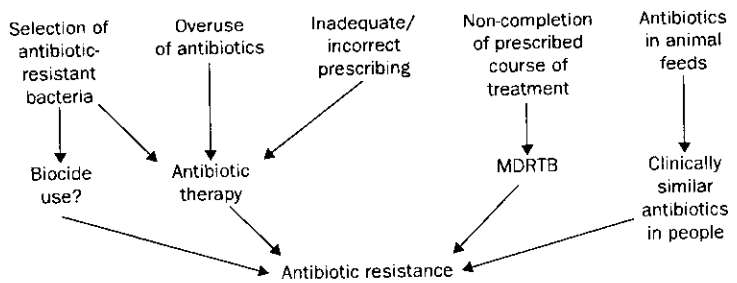

Figure 6. Ways in which antibiotic resistance can arise clinically.

Triclosan-resistant mutants of *S aureus* do not show increased resistance to antibiotics,[18] but MRSA strains trained to QAC resistance show increased resistance to several β-lactam antibiotics.[58] In *E coli*, overexpression of *marA*, *soxS*, or *acrAB* in laboratory or clinical strains reduces their susceptibility to triclosan and also to fluoroquinolones, ampicillin, and tetracycline.[69] Exposure to triclosan of a triclosan-sensitive mutant of *P aeruginosa* switches on an efflux pump that renders the cells highly resistant to ciprofloxacin.[13]

The action of isoniazid against *M tuberculosis* is that of a pro-drug activated by a *katG*-encoded catalase-peroxidase.[70] A protein target, an enoyl reductase (InhA) encoded by the *inhA* gene, is involved in mycolic acid biosynthesis. Mutations in the *inhA* gene in *Mycobacterium smegmatis* result in resistance to triclosan and isoniazid.[26] Mutants selected by triclosan showed increased isoniazid resistance. However, low-level resistance to isoniazid in *M tuberculosis* is associated with point mutations or short deletions within the *katG* gene and high resistance with major deletions in the gene with the loss of all enzyme activity.[71]

Clinical and industrial isolates

Benzalkonium chloride-insusceptible staphylococci are more resistant than sensitive ones to some antibiotics,[72] from which it has been suggested that QAC-containing preparations could be the driving force for selection of bacterial strains resistant to antibiotics in animals. A link has been claimed between insusceptibility to QACs and dyes and resistance to ampicillin and penicillin in clinical isolates of human and animal origin and food-related staphylococci.[72–75] Several workers have shown the low-level resistance (MIC increase 2–8 times) of MRSA strains to cationic biocides.[73,76,77] However, in very few instances have lethal effects been studied. In fact, Cookson et al[78] wondered whether true resistance to chlorhexidine was occurring in hospital MRSA isolates or merely an increased MIC.

The *qac* genes have been widely studied. Several different types (*qacA, B, g, H,* and *smr*) are known.[11] They confer low-level resistance to cationic biocides and may also be associated with antibiotic resistance. However, the *qacA* gene is not seen in antiseptic-sensitive strains.[79] From an ecological and epidemiological point of view the spread of resistant staphylococci in hospitals is claimed to be enhanced by the use of either antibiotics or antiseptics.[80,81] The *qacA* and related genes might have evolved from pre-existing genes responsible for normal cellular processes. The extensive homology between eukaryotic and prokaryotic systems suggests a common ancestry that predates the use of cationic biocides.[82] It has been proposed[82] that (1) *qacA* has evolved from *qacB*, (2) the extensive use of chlorhexidine (and, surprisingly, pentamidine) was responsible for the emergence of the *qacA* determinant, (3) a QAC, benzalkonium chloride, induced the expression of *qacA* and *qacB*, and (4) their chronological emergence in clinical isolates of *S aureus* mirrored the introduction and use of cationic biocides in hospitals. The *qacA* and Tn-related β-lactamase genes in multidrug-resistant *Staphylococcus haemolyticus* have more than 99% identities at the nucleotide stage with the same genes from *S aureus*.[76]

Triclosan has been widely used in skin-care products for 30 years.[83] It is also used as surgical scrubs, handwashes and body washes, and in dental-care products. Its widespread use has led to concerns that it could exert a selective pressure for antibiotic-resistant strains of staphylococci or other bacteria arising in hospital and domiciliary environments.[4] Sensitivity of MRSA isolates to triclosan has changed little over a 10-year period.[21] In this study, more than 230 clinical isolates, including 14 different clones, most of which were EMRSA-15 and EMRSA-16, were studied. These two dominant UK epidemic strains were particularly sensitive to triclosan (MIC_{90} values being 0·06 and 0·03 mg/L, respectively, with the range for both being 0·015–0·25 mg/L).

Furthermore, there is no convincing evidence that triclosan use has resulted in the clinical development of isoniazid-resistant *M tuberculosis*, antibiotic-resistant staphylococci, or antibiotic-resistant Gram-negative bacteria.[13,26] The in-vitro findings of Chuanchen et al[13] with *P aeruginosa*, referred to above, involving a triclosan-sensitive mutant that switched on an efflux pump producing ciprofloxacin resistance has not been seen in practice. Notably, from the same laboratory, there did not seem to be any evidence of crossresistance between triclosan and ciprofloxacin in veterinary strains of this organism.[84]

The widespread use of cationic biocides can result in the selection of Gram-negative bacteria (*P aeruginosa*, *Providencia stuartii*, and *Proteus* spp) that are not only intrinsically insusceptible to these biocides but are also highly resistant to several chemically unrelated antibiotics.[85] The possibility of this unwanted outcome has re-emerged with the suggestion that chlorhexidine could be incorporated into urinary catheters to prevent biofilm formation. This suggestion has met with strong opposition because of the likelihood of antibiotic-resistant Gram-negative bacteria arising.[86] Non-fermenting Gram-negative bacteria (NFGNB, including *Acinetobacter baumannii*, *P aeruginosa*) are resistant to chlorhexidine and to several antibiotics (β-lactams, 4-quinolones, AGACs),[87] which has led to the suggestion that a selective pressure due to the use of low concentrations of chlorhexidine may select strains that are more resistant to antibiotics, thereby increasing the overall level of drug resistance. This conclusion must be viewed with caution.

Correlation analysis attempts to show the possible existence of a linear relation between different groups that

have a measurable output such as sensitivity. It can provide evidence of a relation between, for example, pairings of antibacterial agents, but does not necessarily prove cause and effect.[88] There could be a correlation between increased sensitivity or increased resistance. A negative correlation suggests that resistance to one antibacterial agent of the pair under investigation correlates with sensitivity to the other, whereas a non-significant correlation suggests no specific linear relation between the two agents. Clinical isolates of *P aeruginosa* were generally more resistant to antibiotics than isolates from industrial environments, with antibiotic/biocide correlations occurring especially with the former strains.[89] From this, it was deduced that it was the selective pressure of antibiotic use in the hospital environment that differentiated the clinical environment from the industrial one. For non-genetic resistance to occur between antibiotics and biocides, a bacterial cell must possess a common mechanism of resistance to both types of agent.[89] Adaptive resistance of *P aeruginosa* to amikacin and tobramycin was accompanied by a low-level increase in tolerance to a QAC, benzalkonium chloride.

A recent survey compared the antibiotic and biocide susceptibility (by MRSA testing alone) of MRSA and *P aeruginosa* strains over a 10-year period.[90] The conclusions reached, based on correlation analysis, were that (1) similar families of antimicrobial agents, based on mechanisms of action, grouped together, (2) there was no relation between triclosan (or QACs) and antibiotic resistance, and (3) there were many negative correlations between antibiotics and biocides. Marshall et al[91] could find no significant differences in the overall titres of bacteria, potential pathogens, or frequencies of antibiotic resistance in a single-time analysis of homes using or not using surface antibacterial agents.

Bacterial isolates have been cultured from industrial plants where triclosan and parachlorometaxylenol were manufactured.[92] As expected, *P aeruginosa* isolates were highly insusceptible to both biocides whereas *S aureus* strains were highly sensitive. One strain, *Acinetobacter johnsonii*, isolated from the triclosan plant, was highly insusceptible to triclosan but lost insusceptibility when subcultured in nutrient media without triclosan—ie, in the absence of the selective pressure.

Persistent exposure of bacteria to subinhibitory concentrations of biocides (present as residues)[8] or antibiotics could result in the development and persistence of a low-level insusceptible population which in turn could produce a higher, stable level of insusceptibility.[11] A possible link between biocide and antibiotic resistance may be shown in the laboratory with pure cultures. Studies so far show that no link has yet been seen between continuous use of biocides in communities and an increase in antibiotic resistance.[90,91,93]

Fitness of cells

In general terms, the notion of fitness applies to the average survival and reproduction of individual cells within a phenotype or genotype. Chance events mean that even two apparently identical individuals can differ in their survival and reproduction rates.[94] Mutations can produce reduced or increased fitness or have no effect. When bacteria develop resistance or tolerance, there is often a cost to the cells in terms of fitness and they grow more slowly. Resistance determinants that interfere with normal physiological processes usually cause a reduction in biological fitness[95] that may only be of a short duration with antibiotic-resistant bacteria in vitro and in vivo.[96] Examples occur with plasmid-acquired strains,[97] in antibiotic-resistant *E coli*,[98] and in drug-resistant *M tuberculosis* and *Streptococcus pneumoniae*.[96,99] Bacteria can adapt to this deficit (reduced fitness) under artificial laboratory conditions of serial passage (training) and also in vivo.

Adaptive mutation is defined as the establishment of bacteria encountering foreign environments.[100] Newly evolving activities can confer increased fitness on cells during periods of intense competition.[101] Once adaptation to antibiotic resistance has taken place, resistance remains because there is no disadvantage to the cell in being resistant.[96] The evolutionary nature of antibiotic resistance and its possible relation to biocide use needs to be explored further.[102,103] Thus, although selection of antibiotic-resistant bacteria can occur but be lost when the selective agent is removed,[101,105] changes in prescribing habits alone will not overcome antibiotic resistance.[96]

Bacterial fitness to biocides and the possible costs to the cell have not been widely studied, although it is known that strains adapted to biocide resistance under laboratory conditions may grow more slowly than parent susceptible strains.[106] In practice, resistant clones may be able to survive, certainly within biofilms, and by analogy with antibiotics,[96] bacteria may pay a physiological cost for reduced biocide susceptibility but could survive in the environment until they recover their fitness.

Conclusions

In the laboratory, bacterial exposure to biocides may lead to the induction or development of a coping mechanism that is responsible for crossresistance to certain antibiotics. Such studies are usually done with planktonic cells and near ideal

Future Issues

Additional information is needed about:

- the fitness of bacteria that show reduced susceptibility to biocides under both laboratory and environmental conditions
- hospital disinfection policies[3,4]
- multidrug resistance,[109–111] especially relating to the intensity of biocide use and antibiotic resistance in ICUs,[11] other hospital areas,[110,112,113,114] and other (domiciliary, environmental) situations[15,44,114–116]
- triclosan-containing domiciliary devices and antibiotic resistance[117,118]
- medical devices impregnated with antiseptics or antibiotics and antibiotic resistance[119]
- the possible role of pesticides[120] and other chemical agents[78] in selecting for antibiotic resistance
- the possible association of low-level biocide resistance[20–22] with antibiotic resistance
- the effects of cosmetics[123] and skin antiseptics[124,125] on skin flora and whether there are changes in antibiotic susceptibility
- biocide residues in the environment and biocide and antibiotic resistance[126–128]
- the mechanisms of biofilm resistance to biocides and antibiotics under laboratory and real-life situations[129–137]
- the mechanisms of bacterial insusceptibility and reduced susceptibility to biocides,[138] including enzymatic inactivation[139]
- the mechanisms of biocide action at low and high concentrations[140]

growth conditions. Biofilms, for several complex reasons, provide a mechanism that allows bacterial cells to seem to be less susceptible to a biocide, although on removal of the cells from the matrix, it can be shown that they are equally susceptible to that biocide. In nature, bacteria are frequently seen in both planktonic and biofilm communities, which affects the overall activity of a biocide and the ability of a cell to develop or induce coping mechanisms. Consequently, while biocide use may lead to antibiotic resistance in the laboratory, it does not necessarily equate to the development of such resistance in the natural or clinical environment.

Several studies exploring biocide use and biocide and antibiotic resistance in natural environments—such as drains, clinics, and factories—have been undertaken. Resistant bacteria were not seen in greater numbers in areas where biocides had been employed than in areas where they had not been used. When used correctly, biocides have had and will continue to have an important role in controlling infectious diseases.[107] Future issues that need to be considered are shown in the panel.

Search strategy and selection criteria

Data for this review were identified by searches of Medline and Current Contents over the past 10 years together with the extensive published material in the author's possession in the fields of antimicrobial chemotherapy, biocides, resistance mechanisms and infectious disease control. Keywords used were "bacterial resistance", "biocides", "biocide usage", biocide resistance", and "antibiotic resistance".

Acknowledgments

I thank the Soap and Detergent Association and the Cosmetic, Toiletry, and Fragrance Association, both of Washington, DC, USA for financial support and for reviewing the manuscript before submission. However, the views expressed in this paper are those of the author alone and do not necessarily reflect those of others.

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